

WHAT IS CLAIMED IS:

1. A method for modulating a cellular process, said method comprising contacting a cell in culture under suitable conditions with a cell process-modifying molecule attached to a translocating polypeptide, whereby the cell process-modifying molecule is translocated into the cell in culture and interacts specifically therein with a target site responsive to the cell process-modifying molecule, thereby modulating a cellular process in the cell in culture.
2. A method for transfecting a cell in culture with a target gene, said method comprising contacting the cell in culture under suitable conditions with a polynucleotide comprising the target gene attached to a translocating polypeptide, whereby the cell in culture is transfected by the target gene.
3. The method according to claim 2 wherein the translocating polypeptide is a VP22 polypeptide, Antp, or Protein H.
4. The method according to claim 2 wherein the translocating polypeptide is a VP22 polypeptide and the polynucleotide is translocated into the nucleus of the cell in culture.
5. The method according to claim 2 wherein the polynucleotide is linear or circular DNA containing a cloned open reading frame that encodes the target gene.
6. The method according to claim 5 wherein the polynucleotide is a supercoiled plasmid.
7. The method according to claim 2 wherein the translocating polypeptide is attached to a DNA binding protein and the DNA binding protein links the translocating polypeptide to the polynucleotide.

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8. The method according to claim 7 wherein the DNA binding protein is histone 1 protein, high mobility group 17 protein (HMG17), a polylysine sequence, or an oligopeptide having at least three LARL repeats therein.

9. The method according to claim 2 wherein the translocating polypeptide is attached to a nuclear export signal and the polynucleotide is transfected into the cytoplasm as well as the nucleus of the cell in culture.

10. The method according to claim 9 wherein the nuclear export signal is derived from the HIV Rev protein or the heat stable inhibitor of cAPK.

11. The method according to claim 2 wherein the target gene is stably integrated into the genome of the cell in culture.

12. A method for modulating expression of a target gene product in a cell in culture that contains a target gene under control of one or more regulatory elements, said method comprising contacting the cell in culture under suitable conditions with one or more regulatory agents attached to a translocating polypeptide, whereby the one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements, thereby modulating expression of the target gene product by the cell.

13. The method according to claim 12 wherein the cell in culture is a mammalian, yeast, insect or plant cell.

14. The method according to claim 12 wherein the translocating polypeptide has the properties of:

resistance to proteolysis,
receptor-independent penetration of cell membranes, and
energy-free penetration of cell membranes.

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15. The method according to claim 12 wherein the translocating polypeptide is a VP22 polypeptide, Antp, or Protein H.
16. The method according to claim 12 wherein the translocating polypeptide is a VP22 polypeptide.
17. The method according to claim 12 wherein the regulatory agent is a polynucleotide, a protein or polypeptide, or a small molecule.
18. The method according to claim 12 wherein the cell in culture is transfected with a polynucleotide comprising the target gene.
19. The method according to claim 14 wherein the regulatory element is a promoter and translocation of the regulatory agent transactivates expression of the target gene product by the promoter.
20. The method according to claim 19 wherein the regulatory agent is specific for the promoter.
21. The method according to claim 20 wherein the regulatory agent is a polymerase specific for the promoter.
22. The method according to claim 21 wherein the polymerase is T7 RNA polymerase and the promoter is a T7 promoter.
23. The method according to claim 12 wherein the regulatory agent is an HIV Rev protein and the regulatory element is the HIV Rev response element (RRE).
24. The method according to claim 12 wherein the regulatory agent is a transcription factor specific for the regulatory element and translocation of the regulatory agent transactivates expression of the target gene product.

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25. The method according to claim 12 wherein the regulatory agent and the translocating polypeptide are covalently attached.

26. The method according to claim 12 wherein the regulatory agent and the translocating polypeptide are attached by a linker.

27. The method according to claim 26 wherein the linker comprises one or more disulfide bonds, salicylhydroxamic acid (SHA), phenylboronic acid (PBA), a SHA-NHS ester, or a combination thereof.

28. The method according to claim 12 wherein the translocating polypeptide and the regulatory agent are units of a fusion protein.

29. The method according to claim 12 wherein the regulatory agent is a single chain antibody (sFv).

30. The method according to claim 12 wherein the regulatory agent is a polynucleotide encoding a single chain antibody.

31. The method according to claim 12 wherein the translocating polypeptide and the regulatory agent are covalently linked by a biotin-streptavidin complex or the *E. Coli* single stranded DNA binding protein.

32. The method according to claim 12 wherein the cell line contains a single genomic recombination site and a plasmid containing the target gene and a recombination site that pairs with the genomic recombination site, and wherein the one or more regulatory agents includes a recombinase specific for the paired recombination sites, and wherein translocation of the recombinase causes recombination between the paired recombination sites resulting in stable integration of the target gene into the genome of the cell at the genomic recombinase site.

33. The method according to claim 32 wherein the recombinase is Flp and the recombinase sites are *frt* recombination sites.

34. The method according to claim 32 wherein the recombinase is Cre and the recombinase sites are *lox* recombination sites.

35. The method according to claim 12 wherein the one or more regulatory elements includes a transcription-blocking sequence flanked by recombinase recombination sites and the regulatory agent is a recombinase specific for the recombination sites, wherein translocation of the recombinase causes recombination of the recombination sites, thereby modulating expression of the target gene product.

36. The method according to claim 35 wherein the recombinase recombination sites are *frt* sites and the recombinase is Flp or the recombinase recombination sites are *lox* sites and the recombinase is Cre.

37. The method according to claim 12 wherein the one or more regulatory agents include a single chain antibody specific for a component of the one or more regulatory elements, wherein translocation of the single chain antibody in to the cell and binding of the antibody to the component modulates expression of the target gene product.

38. The method according to claim 12 wherein the target gene is a reporter gene.

39. The method according to claim 12 wherein the target gene is contained within a polynucleotide that further encodes a protein tag.

40. The method according to claim 12 wherein the target gene encodes a toxic protein.

41. The method according to claim 39 wherein the protein tag is a myc epitope, a fluorescent peptide, or a poly His tag, or a combination of any two or more thereof.

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42. The method according to claim 12 wherein the contacting comprises mixing the mammalian or insect cell with an additional cell transfected with a polynucleotide that encodes the regulatory agent and the translocating polypeptide and the additional cell expresses the nucleotide to obtain the regulatory agent attached to the translocating polypeptide.

43. The method according to claim 42 wherein the additional cell is prokaryotic or eukaryotic.

44. The method according to claim 12 wherein the contacting involves incubating the cell line with a soluble protein lysate prepared from an additional transfected cell that expresses one or more polynucleotides encoding the regulatory agent and the translocating polypeptide.

45. The method according to claim 44 wherein the regulatory agent and the translocating polypeptide are expressed by the additional cell as a fusion protein.

46. The method according to claim 12 wherein the cell is refractory to other transfection techniques.

47. The method according to claim 12 wherein the cell is a member of a cell population and expression of the target gene is induced in substantially all of the cell population.

48. A vector comprising a polynucleotide encoding a cell process-modifying molecule attached to a translocating polypeptide.

49. The vector according to claim 48 wherein the vector is has a nucleotide sequence according to SEQ ID NO:1.

50. The vector according to claim 48 wherein the vector is has a nucleotide sequence according to SEQ ID NO:2

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